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Acute waterborne nickel toxicity in the rainbow trout (*Oncorhynchus mykiss*) occurs by a respiratory rather than ionoregulatory mechanism

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Abstract

The acute mechanism of toxicity of waterborne nickel (Ni) was investigated in the rainbow trout (*Oncorhynchus mykiss*) in moderately hard (~ 140 mg l^{-1} as $CaCO_3$) Lake Ontario water, where the 96-h LC_{50} for juvenile trout (1.5–3.5 g) was 15.3 mg (12.7–19.0, 95% C.L.) dissolved Ni l^{-1}. No marked impact of Ni exposure on average unidirectional or net fluxes of Na^+, Cl^-, or Ca^{2+} was observed in juvenile trout exposed for 48–60 h to 15.6 mg Ni l^{-1} as $NiSO_4$. Furthermore, when adult rainbow trout (200–340 g) were fitted with indwelling dorsal aortic catheters and exposed for 117 h to 11.6 mg Ni l^{-1} as $NiSO_4$, plasma ions (Na^+, Cl^-, Ca^{2+}, and Mg^{2+}) were all well conserved. However, mean arterial oxygen tension dropped gradually to ~35% of control values. This drop in P_{aO_2} was accompanied by an acidosis primarily of respiratory origin. P_{aCO_2} rose to more than double control values with a concomitant drop in arterial pH of 0.15 units. Acute respiratory toxicity was further evidenced by a significant increase in hematocrit (Ht), and plasma lactate, and a significant decrease in spleen hemoglobin (Hb). Following 117 h of exposure to 11.6 mg Ni l^{-1}, the gill, intestine, plasma, kidney, stomach, and heart accumulated Ni significantly, with increases of 60, 34, 28, 11, 8, and 3-fold, respectively. Brain, white muscle, liver, and bile did not significantly accumulate Ni. Plasma Ni exhibited a remarkable linear increase with time to levels approximately 30-fold higher than controls. We conclude that in contrast to most other metals, Ni is primarily a respiratory, rather than an ionoregulatory, toxicant at exposure levels close to the 96-h LC_{50}. The implications of a waterborne metal as an acute respiratory toxicant (as opposed to ionoregulatory toxicants such as Cu, Ag, Cd, or Zn) with respect to toxicity modeling are discussed.

Keywords: Nickel; Rainbow trout; Waterborne; Respiratory toxicity; Ionoregulation

1. Introduction

Nickel is ubiquitous in the biosphere and is a common component of natural fresh waters due to erosion and weathering (Eisler, 1998). Fresh water levels of Ni are typically about 1–10 μg l^{-1} in

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unimpacted areas (USEPA, 1980; Eisler, 1998; Chau and Kulikovsky-Cordeiro, 1995), although particular sites (e.g. Smoking Hills, NWT, Canada) may have Ni concentrations several orders of magnitude higher due to natural volcanic processes (Chau and Kulikovsky-Cordeiro, 1995). Anthropogenic loading of Ni into aquatic and terrestrial ecosystems in industrial areas occurs by mining, smelting, refining, alloy processing, scrap metal reprocessing, fossil fuel combustion, and waste incineration (NAS, 1975; WHO, 1991; Chau and Kulikovsky-Cordeiro, 1995; Eisler, 1998). Ni concentrations in highly contaminated fresh waters may reach as high as several hundred to 1000 μg l^{-1} (Eisler, 1998).

Nickel is a transition metal that is generally considered to be an essential micronutrient (Nielsen, 1971). It is consistently found in animal tissues at low levels that appear to be well regulated. Both deficiency and excess of Ni have been shown to reduce survival (for reviews see USEPA, 1980; Eisler, 1998) consistent with the action of other micronutrient transition metals such as Cu and Zn (Simkiss and Taylor, 1989).

Despite growing interest in the fate of Ni in the environment, relatively little is known about the mechanistic action(s) of toxicity—particularly in aquatic ecosystems. The carcinogenic action of insoluble Ni compounds (NiO, Ni_3S_2) has been well studied in mammalian systems (Costa and Heck, 1982; Lee et al., 1982; Costa, 1991), while mechanistic work on soluble Ni compounds ($NiCl_2$, $NiSO_4$) has also been limited, to a large extent, to mammalian systems (Clary, 1975; Gitlitz et al., 1975; Kasprzak et al., 1986). Very recent work by Ptashynski and co-authors has focused on the bioavailability and toxicity of dietary Ni in fish (Ptashynski et al., 2001).

Over the past decade, detailed knowledge of the acute toxic mechanisms of several waterborne trace metals (e.g. Cu, Ag) has facilitated the development of sophisticated models to predict the acute toxicity of a given metal across a wide range of species and water chemistries (e.g. the Biotic Ligand Model (BLM), DiToro et al., 2001). Information on the mechanism of action of Ni would facilitate the inclusion of Ni into such modeling exercises as the BLM.

The objective of the present study, therefore, was to determine the mechanism of acute toxicity of Ni in the rainbow trout (*Oncorhynchus mykiss*), a species that has proven to be a very useful model system for understanding the mechanistic effects of many other metals (Wood, 2001). We hypothesized that the potential mechanism might be any of the following: (i) Na^+ (and Cl^-) antagonism by inhibition of Na^+ uptake as exhibited by Cu (Lauren and McDonald, 1986) and Ag (Morgan et al., 1997); (ii) Ca^{2+} antagonism by inhibition of Ca^{2+} uptake as exhibited by Zn (Spry and Wood, 1985) and Cd (Verbost et al., 1989); and (iii) respiratory toxicity and acid/base disturbance as exhibited by Al at moderately acidic pH (Playle et al., 1989).

2. Materials and methods

2.1. Experimental animals

Juvenile (1.5–3.5 g) rainbow trout for use in LC_{50} and flux experiments, and adult rainbow trout (200–340 g) for use in cannulation experiments, were purchased from Humber Springs Trout Farm, Orangeville, ON. Fish were acclimated for at least 2 weeks to aerated, flowing dechlorinated Hamilton tap water from Lake Ontario at 12–14.5 °C and fed a daily 1% ration (relative to body weight) of commercial trout pellets. Water composition was (in mM) $Ca^{2+} \cong 1$, $Mg^{2+} \cong 0.2$, $Na^+ \cong 0.6$, $Cl^- \cong 0.8$, $SO_4^{2-} \cong 0.25$, titratable alkalinity to pH 4.0 $\cong 1.9$, background Ni $\cong 4$ μg l^{-1}, dissolved organic carbon (DOC) $\cong 3$ mg l^{-1}, total hardness (as $CaCO_3$) of approximately 140 mg l^{-1} and pH 7.9–8.0. Fish were starved 72 h prior to and throughout all experiments.

2.2. Ninety-six hour LC_{50} Determination

A 96-h LC_{50} was measured for juvenile rainbow trout in our test water, using the static-renewal method. Juvenile fish ($n = 10$ per tank) were transferred to one of eight 30 l tanks (including 1 control and 7 Ni concentrations, nominally 1, 4, 8, 16, 24, 32 and 48 mg l^{-1}), each containing 20 l of

well-aerated dechlorinated Hamilton tap water and allowed 48 h to acclimate. Daily, 80% of the water was siphoned out and replaced. Tanks were set on a wet table receiving a constant flow of water for temperature control (12–14.5 °C). At time 0, the exposure tanks were spiked with a concentrated stock prepared from $NiCl_2 \cdot 6H_2O$ (Sigma–Aldrich) dissolved in double-distilled water (NANOpure II; Sybron/Barnstead, Boston MA).

The highest Ni concentration used (48 mg l^{-1}) necessitated the addition of approximately 1.6 mmol l^{-1} of Cl^-, effectively tripling background Cl^- levels (0.8 mM) in Hamilton tap water. As a control for possible Cl^- effects, an identical exposure tank and protocol were used with NaCl added to yield a final Cl^- concentration similar to that of the highest Ni exposure tank. There was no mortality in this treatment.

Mortality was monitored daily. Water samples were also taken daily, filtered with a 0.45 μm filter, and analyzed for dissolved Ni by graphite furnace atomic absorption spectrophotometry (GFAAS; 220 SpectrAA; Varian, Australia) against certified atomic absorption standards (Fisher Scientific). In practice, dissolved Ni levels (0.45 μm filtered) and total Ni (unfiltered) levels measured on the same water samples typically differed by less than 5%, and with no predictable pattern of difference. It was, therefore, assumed that the dissolved (filtered) fraction of Ni was equal to total (unfiltered) Ni. The LC_{50} and 95% confidence limits were calculated using the probit method (SPSS), employing mean measured dissolved Ni concentrations in the exposure tanks.

2.3. Ion flux experiments

2.3.1. Exposure conditions

The exposure conditions were identical to those described above for the LC_{50} experiments with three exceptions: (i) $n = 30–35$ per tank, (ii) $NiSO_4 \cdot 6H_2O$ was added, not the chloride salt (as we wished to measure Cl^- fluxes), and (iii) three tanks were used—one control and two replicates of the exposure concentration of 15.6 mg Ni l^{-1}, chosen on the basis of the results of the 96-h LC_{50} test. Again, daily water samples were filtered and analyzed for Ni by GFAAS.

2.3.2. Flux chambers

Unidirectional and net Na^+ and Cl^- fluxes, and unidirectional Ca^{2+} fluxes were measured on control fish at 0 and 60 h, and on experimental fish at 2 (not Ca^{2+}), 4, 8, 12, 24, 36, 48 and 60 h (Ca^{2+} only) of Ni exposure. The flux chambers consisted of 20 ml plastic syringes that were sealed and placed in a shallow wet table receiving a constant flow of water to maintain temperature control. The syringes were covered in such a way as to ensure that the fish were unable to see the experimenter. A small hole pre-drilled into the top of the syringe was fitted with thin tubing for aeration, while a second larger hole in the top served for loading of radioisotope and water sampling. Approximately 0.5 h (Na^+ and Cl^-) or 0.25 h (Ca^{2+}) prior to the appropriate time, fish from the control and exposure tanks were loaded individually into flux chambers filled with water of the appropriate quality (i.e. control or 15.6 mg Ni l^{-1}). At this time, radioisotope was added and allowed to equilibrate for 0.5 h (Na^+ and Cl^-) or 0.25 h (Ca^{2+}) before water sampling. This equilibration period also served as a 'settling' period to calm fish placed in the flux chamber. ^{22}Na (NEN Life Sciences Products, Inc, Boston MA) and ^{36}Cl (ICN Biomaterials, Inc, Irvine CA) were added together in one round of flux experiments at 0.1 μCi each per flux chamber (yielding approximate water specific activities of 8000 and 10 000 cpm μmol^{-1}, respectively), while ^{45}Ca (NEN) was added in a separate round of experiments at 3 μCi per chamber (yielding an approximate water specific activity of 300 000 cpm μmol^{-1}). The addition of radioisotope did not appreciably alter the concentrations of the relevant ions in the water.

No mortality was observed during any flux period, and mortality in control and exposure tanks never exceeded 5%.

2.3.3. Na^+ and Cl^- flux measurements and calculations

Initial and final water samples (1.5 h apart) were taken for determination of ion concentration (1

ml) and radioisotope counting (1 ml). Water sample analyses and calculations of unidirectional and net Na^+ and Cl^- fluxes followed the methods of Wood (1992), and Morgan et al. (1997). ^{22}Na is a dual γ and β emitter, while ^{36}Cl is a pure β emitter. Water samples were first analyzed for ^{22}Na radioactivity by γ counting (Minaxi γ; Canberra–Packard, Meridan, CT), followed by addition of scintillation fluid (10 ml of ACS, Amersham) to the same water samples for ^{36}Cl plus ^{22}Na radioactivity measurement by β counting (1217 Rackbeta, LKB Wallac, Turka, Finland). After correcting for differences in ^{22}Na counting efficiencies in the two instruments, ^{36}Cl radioactivity (alone) was obtained by subtracting the corrected ^{22}Na radioactivity from the total radioactivity measured by scintillation counting. $[Na^+]$ was measured by flame atomic absorption spectrophotometry (FAAS, 220FS SpectrAA, Varian, Australia), while $[Cl^-]$ was analyzed by the mercuric thiocyanate spectrophotometric method (Zall et al., 1956).

The unidirectional influxes of Na^+ or Cl^- (J_{in}^{ion}, μEq kg^{-1} h^{-1}) were calculated by the formula:

$$J_{in}^{ion} = \frac{(cpm_i - cpm_f)V_{ext}}{1/2[cpm_i/[ion]_i + cpm_f/[ion]_f]W \cdot t} \quad (1)$$

where cpm_i and cpm_f are the radioactivity in counts min^{-1} ml^{-1} of initial and final water samples, V_{ext} is the flux volume (ml) corrected for sampling deficits, $[ion]_i$ and $[ion]_f$ are the initial and final concentrations of Na^+ or Cl^- in the flux water, respectively, W is the weight of the fish in grams, and t is the time of the flux period in hours. This method assumes that all radioactivity lost from the water is transferred exclusively to the fish, consistent with the lack of confounding adsorption of monovalent ions such as Na^+ or Cl^- to plastic or fish surfaces (Wood, 1992), and yields average flux rates for the period in question. Additionally, this method assumes that no appreciable amount of radiolabeled ion effluxes from the fish during the 1.5 h flux period. Calculation of internal (fish) specific activity of ^{22}Na and ^{36}Cl at the end of each flux period yielded a value less than 3% of water specific activity, and, therefore, no correction for potential radiotracer efflux was necessary (Kirschner, 1970).

Net fluxes (J_{net}^{ion}) were calculated from:

$$J_{net}^{ion} = \frac{([ion]_i - [ion]_f)V_{ext}}{W \cdot t} \quad (2)$$

where all of the symbols are as described above, and efflux (J_{net}^{ion}) was calculated by the conservation equation:

$$J_{out}^{ion} = J_{net}^{ion} - J_{in}^{ion} \quad (3)$$

Immediately following the flux period, fish were removed from the syringe, euthanized with a blow to the head, blotted dry, weighed to the nearest 0.01 g and placed in a 20 ml scintillation vial. After bodies were digested at 60 °C for 48 h in 1 N HNO_3 (Fisher Scientific; trace metal grade), the digest was homogenized by vortexing, an aliquot removed ($\sim$1.5 ml), centrifuged at 13 000 × g for 10 min and the supernatant diluted for analysis of $[Na^+]$ by FAAS, and $[Cl^-]$ by the mercuric thiocyanate method as described above.

2.3.4. Ca^{2+} influx measurements and calculations

Determination of unidirectional and net fluxes of Ca^{2+} by the method described above is not feasible due to the high adsorption capacity of the fish surface and most other surfaces for Ca^{2+} (Wood, 1992). Therefore, Ca^{2+} influx was determined by measuring the appearance of the radiotracer in the animal. Procedurally, the Ca^{2+} flux experiments were similar to the Na^+ and Cl^- experiments with three exceptions—initial and final water samples for $[Ca^{2+}]$ and for ^{45}Ca radioisotope counting were 800 and 200 μl, respectively, the flux periods were 4 h rather than 1.5 h, and the 0.5 h pre-incubation period was reduced to 0.25 h. At the end of the flux period, fish were removed from the syringe, immediately euthanized with a blow to the head, and rinsed for 1 min in 1 mM EDTA (ethylenediamine-tetraacetic acid; Sigma–Aldrich) followed by 1 min in dechlorinated tap water (1 mM Ca^{2+}) to remove all surface bound ^{45}Ca. Fish were blotted dry, weighed to the nearest 0.01 g, wrapped in foil, and stored at −80 °C.

Fish were ground under liquid nitrogen until homogenized and tissue aliquots in triplicate (~ 100 mg) were transferred to 20 ml glass scintillation vials and thawed to a constant weight. Tissue aliquots were solubilized (1 ml NCS, Amersham) and left at 50 °C in a shaking water bath overnight. The digest was neutralized (~ 30 μl glacial acetic acid, diluted with scintillation fluid (10 ml OCS, Amersham), stored in the dark overnight to reduce chemoluminescence and ^{45}Ca radioactivity measured on a scintillation counter as described above, with the additional step of quench correction to the same counting efficiency as water samples by the method of external standard ratios.

^{45}Ca radioactivity in the exposure water was measured by adding 200 μl water samples to 2 ml of scintillation fluid (ACS, Amersham) and β counting. Water samples for analysis of $[Ca^{2+}]$ were diluted with 0.2% La^{3+} and analyzed by FAAS. Unidirectional calcium influxes ($J_{in}^{Ca^{2+}}$, μEq kg^{-1} h^{-1}), again averages for the period in question, were determined by the formula:

$$J_{in}^{Ca^{2-}} = \frac{\text{cpm } g^{-1} \text{ fish}}{1/2[\text{cpm}_i/[Ca^{2+}]_i + \text{cpm}_f/[Ca^{2+}]_f]t} \quad (4)$$

where cpm_i, cpm_f, and t are as described above, and $[Ca^{2+}]_i$ and $[Ca^{2+}]_f$ are the initial and final calcium concentration in the flux water. Changes in the latter were so small as to preclude the measurement of $J_{net}^{Ca^{2+}}$. Again, calculated internal ^{45}Ca specific activity at the end of the 4 h flux period yielded a value less than 1% of water specific activity, and no correction for radiotracer efflux was necessary (Kirschner, 1970).

2.4. Cannulation experiments

2.4.1. Experimental protocol

Two time series cannulation experiments were run—different only in the concentration used and the sampling times. The first series (A) was run at a Ni concentration of 18.4 mg Ni l^{-1}, with sampling times of 0 (control), 6, 18 and 66 h. The only data shown from this experiment appear in Fig. 4. The remaining cannulation data were collected from the second series (B) at 11.6 mg Ni l^{-1}, with sampling times of 0 (control), 24, 48, 72, 99, and 117 h.

Adult rainbow trout were anaesthetized with MS222 (0.075 g l^{-1} neutralized with NaOH) on an operating table, surgically fitted with indwelling dorsal aortic catheters (Clay Adams PE 50 tubing, Soivio et al., 1972), transferred to individual darkened plexiglass chambers (3 l) served with a water flow of 100 ml min^{-1} and continuous aeration, and allowed to recover for 48 h. Each experiment started with eight control and eight experimental fish bearing functional catheters. Water feeding the control chambers flowed directly from an aerated head tank, while water to the Ni exposure chambers was routed (gravity fed) through a mixing chamber before feeding the individual exposure chambers. Immediately following the control sampling, $NiSO_4 \cdot 6H_2O$ was added by gravity feed from a concentrated stock solution stored in a light shielded stock bottle, into the vigorously aerated mixing chamber. Additionally, at this time, the mixing chamber and all individual exposure chambers were spiked with stock solution to bring them up to measured dissolved Ni concentrations of either 18.4 ± 0.2 mg Ni l^{-1} (A) or 11.6 ± 0.2 mg Ni l^{-1} (B).

The sampling protocol followed closely that of Wood et al. (1996). Daily, each fish was sampled as follows: ventilation rate was counted visually, and then water samples from in front of each fish's mouth were filtered (0.45 μm) and later analyzed for dissolved Ni by GFAAS. Unfiltered water samples were then taken for inspired O_2 tension (P_{IO_2}) and pH_I. Blood (1 ml) was drawn anaerobically via the arterial catheter into an ice-cold, Li-heparanized (50 i.u. ml^{-1}; Sigma–Aldrich), gas-tight Hamilton syringe for analysis of blood pH (pH_a), O_2 tension (P_{aO_2}), plasma total CO_2 (C_{aCO_2}), hematocrit (Ht), blood hemoglobin (Hb), and plasma levels of Na^+, Cl^-, Ca^{2+}, Mg^{2+}, lactate, glucose, protein, cortisol, total ammonia, and Ni. Plasma was separated by centrifugation at $13\,000 \times g$ for 1 min and erythrocytes were gently resuspended in the appropriate volume of Cortland saline (Wolf, 1963) and reinjected into the fish. Three separate plasma aliquots were preserved for the determination of (i) lactate and glucose, (ii) cortisol, and (iii) total

ammonia, electrolyte, and Ni concentrations. The former aliquot was immediately deproteinized in two volumes of ice-cold 6% perchloric acid, while the latter two aliquots were transferred to cryostatic vials, frozen in liquid nitrogen and stored at −80 °C. Prior to analysis, these two aliquots were thawed and sonicated on ice for 5 s at 5 W (Microson; Misonix Inc, Farmingdale, NY, USA).

2.4.2. Analytical methods

Analyses of pH_a, P_{aO_2}, inspired water pH (pH_I) and O_2 tension (P_{IO_2}) employed Radiometer electrodes and meters, and were identical to those of Wood et al. (1988). Hb was determined by the colorimetric cyanmethemoglobin method (Sigma–Aldrich reagents). Plasma for total CO_2 (C_{aCO_2}) and protein concentration was obtained by centrifuging whole blood ($5000 \times g$ for 10 min) in ammonium-heparanized microhematocrit tubes in duplicate. Ht was measured directly from the tubes, while total CO_2 was analyzed on true plasma using a Corning 965 CO_2 analyzer and plasma protein was determined with an American Optical Goldberg refractometer (Alexander and Ingram, 1980). Lactate was measured enzymatically (L-lactate dehydrogenase/NADH at 340 nm; Sigma–Aldrich) on deproteinized plasma, while glucose was measured enzymatically (hexokinase/glucose-6-phosphate dehydrogenase at 340 nm; Sigma–Aldrich) on the same acid extracts neutralized with 1 M K_2CO_3. Plasma cortisol was determined using an ^{125}I radioimmunoassay (ICN Biomedicals; Montreal, Quebec) with radioactivity measured by γ counting. Plasma total ammonia concentration was determined enzymatically (glutamate dehydrogenase/NADP at 340 nm; Sigma–Aldrich), while $[Na^+]$, $[Ca^{2+}]$ and $[Mg^{2+}]$ were determined by FAAS, and [Ni] by GFAAS. Plasma $[Cl^-]$ was measured by the mercuric thiocyanate method described above.

At the end of the experiment, fish were euthanized with an overdose of anesthesia (0.5 g l^{-1} MS 222, neutralized with NaOH), blotted dry and weighed to the nearest 0.1 g. Tissues surgically removed for analysis included liver, kidney, heart, brain, stomach, intestine (plus pyloric caeca), and bile for Ni only, and white muscle and gill filaments for Ni, Na^+, and Cl^-. All tissues were stored at −20 °C. After tissues were digested at 60 °C for 48 h in 1 N HNO_3 (Fisher Scientific; trace metal grade), the digest was homogenized by vortexing, centrifuged at $13\,000 \times g$ for 10 min and the supernatant diluted for analysis of Ni by GFAAS, Na^+ by FAAS, and Cl^- by the mercuric thiocyanate method. The spleen was removed and weighed to the nearest 0.001 g and stored at −20 °C for later analysis of Hb content using the homogenization method of Milligan and Wood (1982) and the colorimetric cyanmethemoglobin assay (Sigma–Aldrich reagents).

2.4.3. Calculations

Calculations of P_{aCO_2} and plasma HCO_3^- based on measured pH_a and C_{aCO_2} were identical to those described in Playle et al. (1989) using the Henderson–Hasselbach equation and values for CO_2 solubility (αCO_2) and apparent pK (pK′) at the appropriate temperature from Boutilier et al. (1984). Mean cellular hemoglobin concentration (MCHC) was calculated as the ratio of simultaneous Hb to Ht and is expressed as grams Hb per ml RBC. Metabolic acid load (ΔH_m^+) was calculated from the equation of McDonald et al. (1980).

$$\Delta H_m^+ = [HCO_3^-]_1 - [HCO_3^-]_2 - \beta(pH_1 - pH_2)$$

where the subscripts 1 and 2 refer to plasma bicarbonate and pH values at daily intervals, and the slope of the true plasma buffer capacity (β) was calculated from the regression relationship between measured whole-blood Hb and β as determined for rainbow trout plasma at this temperature by Wood et al. (1982).

2.5. Statistical analyses

All measured and calculated values are presented as mean ± 1 S.E.M. (n). Where control measurements were made simultaneous to all experimental measurements (i.e. Figs. 4–6, 8–10, cannulation experiments), time-dependent responses in both control and experimental groups were tested against their respective 0 h values by a one-way ANOVA with a two-sided Dunnett's post hoc multiple comparison, and each experimental mean was compared with its simultaneous control mean by an unpaired two-tailed Student's t-test.

Where both initial and final control means were taken so as to temporally bracket experimental means (i.e. Figs. 1–3, flux experiments and associated whole-body ion analysis), it was first confirmed that the two control means were not significantly different (unpaired two-tailed Student's t-test), then time-dependent responses of experimental fish were tested against the mean of all of the control values by a one-way ANOVA with a two-sided Dunnett's post hoc multiple

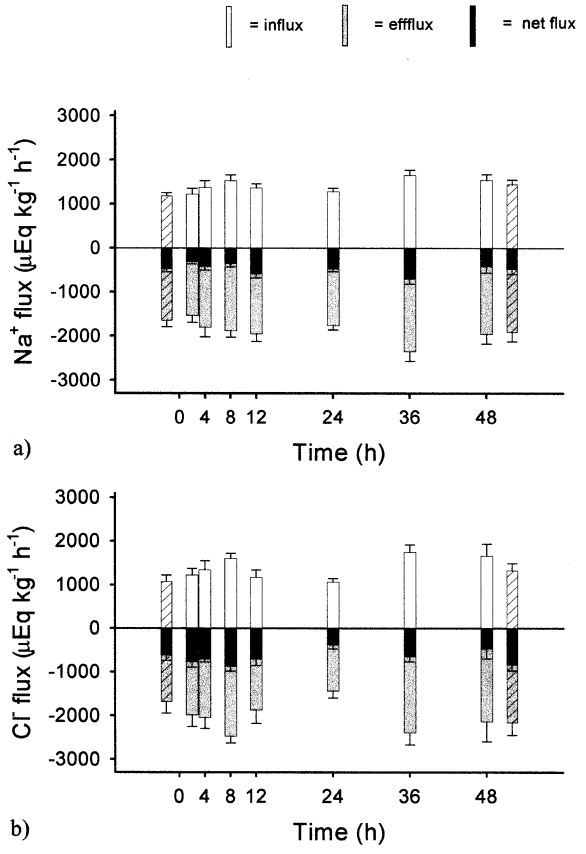

Fig. 1. Whole-body influx (white bars), efflux (gray bars), and net flux (black bars) of Na^+ (a) and Cl^- (b) in juvenile rainbow trout exposed to control conditions or 15.6 mg Ni l^{-1} over 48 h. Two-hour flux periods ended at the time given on the x-axis (see text for more details). Data are expressed as mean ± 1 S.E.M. ($n = 8$). Hatched bars at the beginning and end of the time period represent initial and final control influx measurements, respectively. These were not significantly different ($P >$ 0.05). There were no significant differences ($P > 0.05$, one-way ANOVA plus two-sided Dunnett's post hoc test) from the combined control.

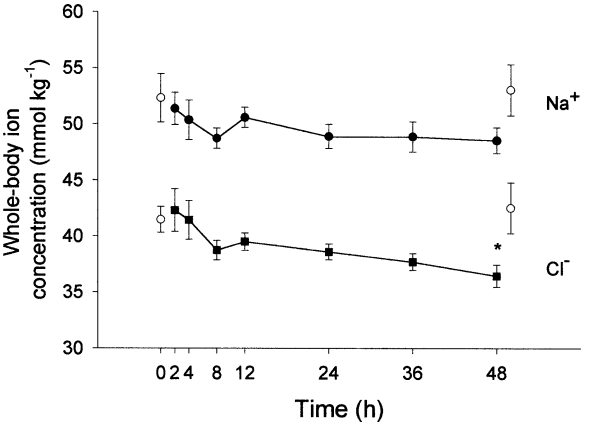

Fig. 2. Whole-body Na^+ and Cl^- concentrations of juvenile rainbow trout exposed to control conditions or 15.6 mg Ni l^{-1} over 48 h. Data are expressed as mean ± 1 S.E.M. ($n = 8$). Control values at 0 and 48 h are given as open circles. These were not significantly different ($P > 0.05$). Asterik '*' indicates significant difference ($P < 0.05$, one-way ANOVA plus two-sided Dunnett's post hoc test) from the combined control.

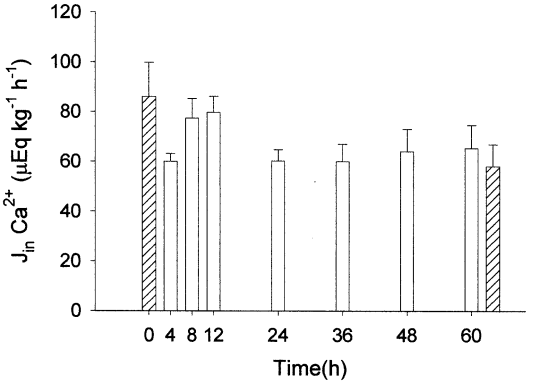

Fig. 3. Whole-body influx of Ca^{2+} in juvenile rainbow trout exposed to control conditions or 15.6 mg Ni l^{-1} over 60 h. About 4 h flux periods ended at the time given on the x-axis (see text for more detail). Data are expressed as mean ± 1 S.E.M. ($n = 8$). Hatched bars at the beginning and end of the time period represent initial and final control influx measurements, respectively. These were not significantly different ($P >$ 0.05). There were no significant differences ($P > 0.05$, one-way ANOVA plus two-sided Dunnett's post hoc test) from the combined control.

comparison. Statistical significance in all cases is given at $P < 0.05$. The reduction in control n during the cannulation experiments resulted from cannula failure, as there was no mortality among

control animals during either cannulation series, whereas, decreases in the *n* of experimental fish were due to a combination of cannula failure and mortality (see Section 3).

3. Results

3.1. Ninety-six hour LC_{50}

A 96-h static-renewal LC_{50} for dissolved Ni of 15.3 mg l^{-1} was calculated with lower and upper 95% confidence limits of 12.7 and 19.0 mg Ni l^{-1}, respectively.

3.2. Na^+ and Cl^- fluxes

Exposure to the approximate 96-h LC_{50} level of dissolved Ni (15.6±0.3 mg l^{-1}) for up to 48 h had no marked effect on either the average unidirectional or net fluxes of Na^+ or Cl^- (Fig. 1), strongly suggesting that a mechanism other than Na^+ and Cl^- ionoregulatory disturbance is the origin of acute toxicity. Indeed, there was no inhibition of either Na^+ or Cl^- unidirectional uptake rate. Furthermore, there was no effect of Ni exposure on either Na^+ or Cl^- unidirectional efflux rates. Net Na^+ and Cl^- balance was negative in all fish at all times (i.e. efflux greater than influx), but this occurred in control fish to the same extent as in experimental fish, and probably reflected the stress of confinement during the flux measurement periods. Importantly, controls run at 0 and 48 h gave virtually identical results, indicating that there was no problem associated with the exposure conditions themselves.

As a check on whole-body Na^+ and Cl^- losses in these fish over the 48 h exposure, ions were measured in the whole body immediately following each flux period (Fig. 2). Note that each fish had been confined in the flux chamber for only 2 h, but had experienced dissolved Ni (15.6±0.3 mg l^{-1}) for the time shown in Fig. 2. Losses of Na^+ and Cl^- over the entire 48 h exposure period amounted to approximately 7 and 10% of whole-body levels, respectively. Only whole-body [Cl^-] at 48 h was significantly depressed. There was no significant change in control fish between 0 and 48 h, so these losses were the result of Ni exposure. However, the overall magnitude of these ion losses was not large enough to indicate severe (lethal) ionoregulatory toxicity (see Section 4).

3.3. Ca^{2+} influx

Average calcium influx rates in juvenile trout were not significantly changed by 60 h of exposure to 15.6±0.3 mg l^{-1} of dissolved Ni (Fig. 3). A non-significant trend towards decreased uptake rates over the 60 h exposure period in experimental fish was mirrored by initial and final influxes of control fish.

3.4. Cannulation experiments

3.4.1. Mortality

In the first series (A) at a dissolved Ni concentration of 18.4±0.2 mg l^{-1}, three of eight experimental animals died by 66 h (when the experiment was terminated), compared with zero of eight in the simultaneous control series. Similarly, in the second series (B) at 11.6±0.2 mg Ni l^{-1}, three of eight experimental animals died by 117 h (when the experiment was terminated), compared with zero of eight in the control series. In the second series, from which most of the data are reported, the most impacted experimental animals died between 99 and 117 h. Therefore, the apparent return of many experimental means toward control levels at 117 h is to be interpreted as a 'survivor effect', and not as apparent recovery. Fig. 6a (inset) illustrates in detail this scenario for plasma lactate measurements. Our focus is, therefore, confined to the first 99 h of exposure with respect to parameters most impacted.

3.4.2. Blood gases and acid/base balance

The first series (A) at 18.4±0.2 mg Ni l^{-1} was designed to provide an initial diagnosis of the toxic mechanism(s) and the time course of toxicity. Between the 18 and 66 h sampling points, there was substantial mortality and marked changes in blood gas parameters—a sharp fall in P_{aO_2} and rise in P_{aCO_2} (Fig. 4)—making it difficult to resolve patterns in detail using this exposure concentration and sampling regime. Other re-

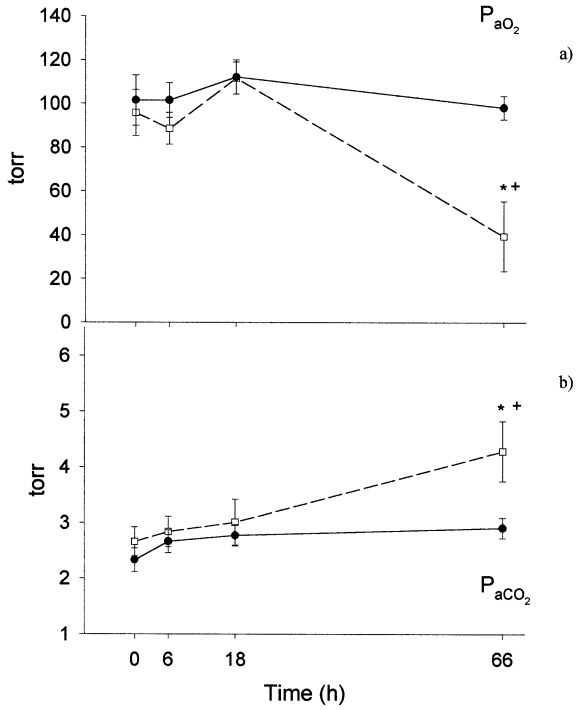

Fig. 4. Arterial blood gases (P_{aO_2} and P_{aCO_2}) obtained by cannulation of adult rainbow trout exposed to control conditions or 18.4 mg Ni l^{-1} over 66 h. Note that the exposure concentration and sampling regime of this cannulation series (A) differ from those of Figs. 5–9 and 5 (Series B). Data are expressed as mean (±1 S.E.M.) ($n = 6$–8 control; $n = 5$–8 experimental). Asterisk '*' indicates significant difference ($P < 0.05$; one-way ANOVA plus two-sided Dunnett's post hoc test) from time 0 h mean. '+' indicates significant difference ($P < 0.05$; two-tailed Student's t-test) from simultaneous control mean.

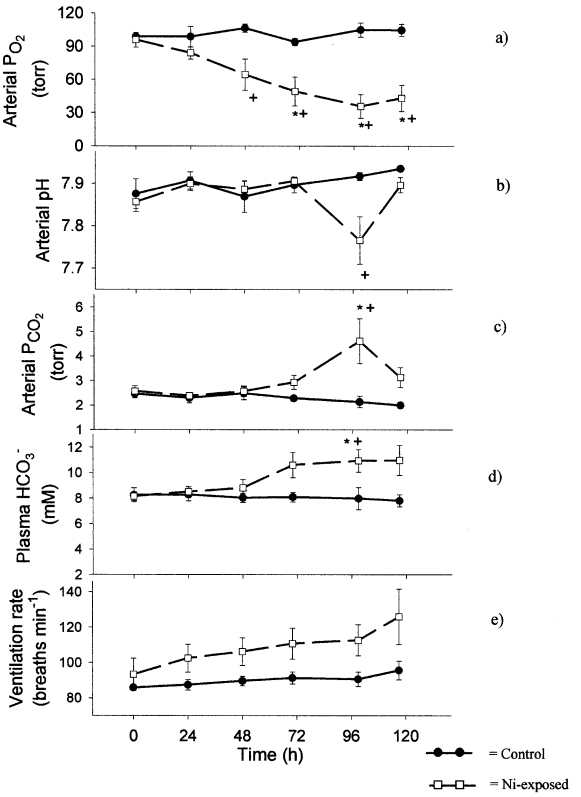

Fig. 5. Arterial blood gases and acid/base parameters obtained by cannulation of adult rainbow trout exposed to control conditions or 11.6 mg Ni l^{-1} over 117 h. (a) Arterial oxygen tension (P_{aO_2}), (b) arterial blood pH (pH_a), (c) arterial plasma carbon dioxide tension (P_{aCO_2}), (d) arterial plasma [HCO_3^-], (e) ventilation rate. Data are expressed as mean ±1 S.E.M. ($n = 5$–8 control; $n = 4$–8 experimental). Asterisk '*' indicates significant difference ($P < 0.05$, one-way ANOVA plus two-sided Dunnett's post hoc test) from time 0 h mean. '+' Indicates significant difference ($P < 0.05$, two-tailed Student's t-test) from simultaneous control mean.

sponses were qualitatively similar to those in the second series (B). Based on the time course of the most impacted parameters, we decided to reduce the exposure concentration to 11.6 ± 0.2 mg Ni l^{-1} in series B and sample daily with the goal of obtaining a more detailed analysis of the cause of death.

In the second series, acute respiratory toxicity was again observed (Fig. 5). Over 99 h, mean P_{aO_2} dropped almost linearly with time to 35% of control values (Fig. 5a), with the decline first becoming significant at 48 h. P_{aO_2} values in moribund fish were often less than 20 torr (not shown). Water P_{IO_2} remained constant between 130 and 150 torr (not shown) and water pH_I remained constant between 7.8 and 8.1 (not shown). This severe drop in P_{aO_2} was accompanied by an acidosis (Fig. 5b) of predominantly respiratory origin. By 99 h, P_{aCO_2} had risen to more than double control values (Fig. 5c) with a concomitant drop in arterial pH of 0.15 units (Fig. 5b), reflecting a severe impairment of gas exchange. Arterial pH appeared to be stabilized until 72 h by a compensatory increase in plasma HCO_3^- concentration (Fig. 5d), but thereafter declined. Ventilation rate increased non-significantly over 99 h

in Ni-exposed fish (Fig. 5e) and though not quantified, there was also a noticeable increase in ventilatory stroke volume.

No substantial contribution of metabolic (non-volatile) acids to the blood acid load (ΔH_m^+) was observed over 99 h of Ni exposure (Fig. 6b), despite a significant tripling of plasma lactate at 99 h (Fig. 6a). Fig. 6a inset illustrates the dichotomous pattern of the 'survivor effect'.

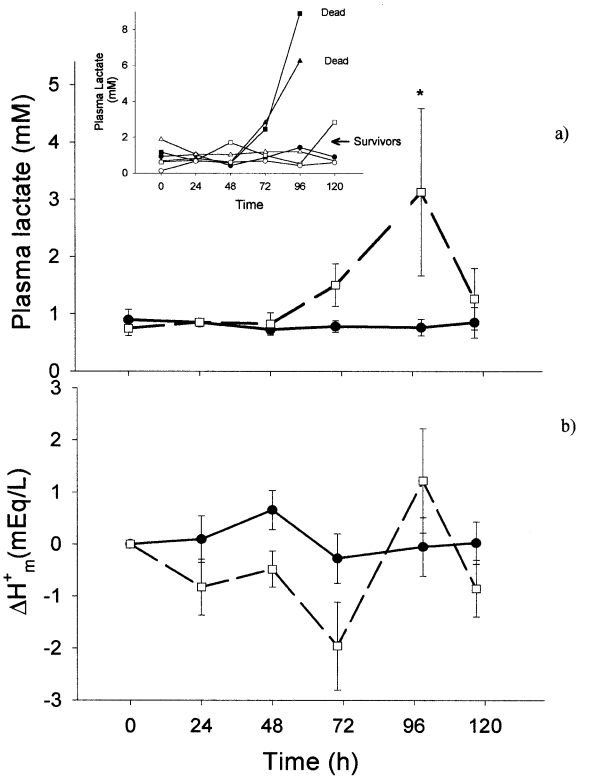

Fig. 6. (a) Arterial plasma [lactate] and (b) net daily metabolic acid accumulation in blood (ΔH_m^+) obtained by cannulation of adult rainbow trout exposed to control conditions or 11.6 mg Ni l^{-1} over 117 h. Data are expressed as mean ± 1 S.E.M. (n = 5–8 control, n = 4–8 experimental). Asterisk '*' indicates significant difference ($P < 0.05$, one-way ANOVA plus two-sided Dunnett's post hoc test) from time 0 h mean. Inset: evidence of a 'survivor effect' among cannulated experimental animals exposed to 11.6 mg Ni l^{-1}. Plasma lactate concentrations of six individual Ni-exposed fish are plotted. Of the six fish plotted through 99 h, the two most impacted (~ 6 and 9 mM) died before the 117 h sampling, causing the appearance of recovery of the mean at 117 h. Note that one of the surviving fish has an escalating plasma lactate concentration (~ 3 mM) at 117 h.

3.4.3. Stress indicators

Plasma cortisol (by 72 h) and total ammonia (by 99 h) had increased several-fold over control values in experimental fish, though substantial variation precluded statistical significance in the case of total ammonia (Fig. 7a and b). Plasma glucose had begun to rise slightly at 99 h, though not significantly (Fig. 7c).

3.4.4. Plasma ion and Ni levels

Plasma levels of Na^+, Cl^-, Ca^{2+} and Mg^{2+} were minimally impacted over 99 h of Ni exposure (Fig. 8a–d), providing additional support that a

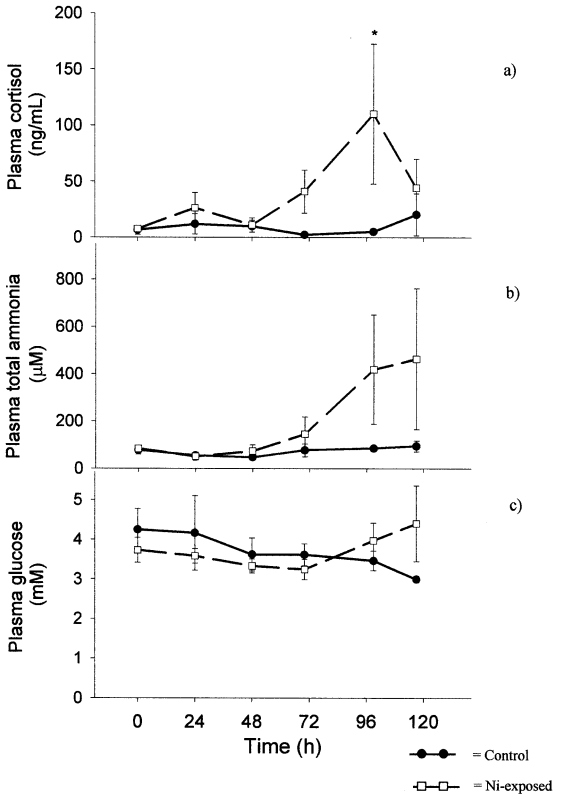

Fig. 7. Stress indices in arterial blood plasma obtained by cannulation of adult rainbow trout exposed to control or 11.6 mg Ni l^{-1} over 117 h. (a) Arterial plasma [cortisol], (b) arterial plasma total ammonia, (c) arterial plasma [glucose]. Data are expressed as mean ± 1 S.E.M. (n = 5–8 control, n = 4–8 experimental). Asterisk '*' indicates significant difference ($P < 0.05$, one-way ANOVA plus two-sided Dunnett's post hoc test) from time 0 h mean.

mechanism other than ionoregulatory disturbance is the origin of acute toxicity. Na^+ and Ca^{2+} were virtually unaffected, while Cl^- (99 h) and Mg^{2+} (from 24 h onward) were slightly, but not significantly, lower in experimental fish.

Nickel-exposed fish were able to conserve appropriate plasma Na^+ and Cl^- levels (Fig. 8a and b) without severe consequences to tissue ion homeostasis, as no significant differences were found in gill or white muscle Na^+ or Cl^- (Table 1). Nickel exhibited a remarkable build-up in plasma, accumulating linearly over time from background control levels of 250 μg l^{-1} at time 0 h to approximately 6300 μg l^{-1} by 99 h, though this was still below the level (11 600 μg l^{-1}) in the exposure water (Fig. 8e).

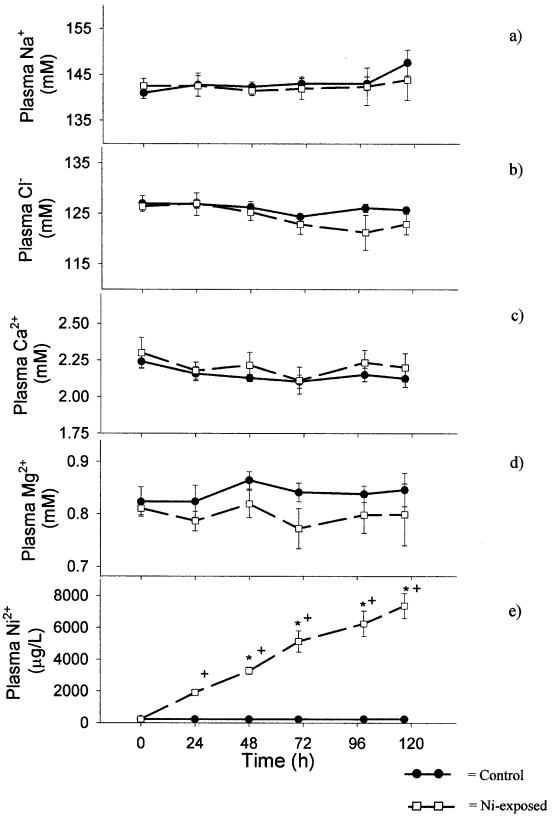

Fig. 8. Arterial plasma ion concentrations of Na^+ (a), Cl^- (b), Ca^{2+} (c), Mg^{2+} (d), and Ni (e) obtained by cannulation of adult rainbow trout exposed to control conditions or 11.6 mg Ni l^{-1} over 117 h. Data are expressed as mean ± 1 S.E.M. (n = 5–8 control; n = 4–8 experimental). Asterisk '*' indicates significant difference ($P < 0.05$; one-way ANOVA plus two-sided Dunnett's post hoc test) from time 0 h mean. '+' Indicates significant difference ($P < 0.05$; two-tailed Student's t-test) from simultaneous control mean.

Table 1
$[Na^+]$ and $[Cl^-]$ in gill and white muscle of terminally sampled fish following exposure to control or 11.6 mg Ni l^{-1} for 117 h

	Control (mmol kg^{-1})	Ni-exposed (mmol kg^{-1})
Gill Na^+	77.11 ± 1.40	76.39 ± 2.39
Gill Cl^-	92.21 ± 2.30	90.67 ± 2.60
White muscle Na^+	22.10 ± 1.30	25.40 ± 1.21
White muscle Cl^-	35.27 ± 1.65	34.92 ± 0.71

Control $n = 9$; experimental $n = 6$. There were no significant differences ($P > 0.05$). Data are expressed as mean ± 1 S.E.M.

3.4.5. Hematology

Due to repetitive blood sampling, hematocrit (Ht) and hemoglobin (Hb) fell over time in the control group, but not in Ni-exposed fish where Ht and Hb were almost perfectly conserved. Ht was significantly greater in Ni-exposed fish from 72 h onward (Fig. 9a), while Hb followed a very similar, though non-significant trend (Fig. 9b). Thus, the lack of change in Ht and Hb in Ni-exposed fish represents 'increases' in these hematological parameters. There was no change in MCHC (Fig. 9c), indicating that erythrocytic swelling did not occur. Plasma protein concentration did not change significantly in either group, though by 99 h, it was 22% higher in the Ni-exposed fish relative to the control group (Fig. 9d).

Terminal spleen hemoglobin (g Hb g^{-1} spleen) in Ni-exposed fish was significantly reduced by 30% (0.061 ± 0.005 in control fish vs. 0.042 ± 0.003 in Ni-exposed fish), indicating splenic discharge.

3.4.6. Ni accumulation

Accumulation of Ni in various tissues of fish sampled at 117 h of exposure, along with a comparison of water Ni and plasma Ni levels is given in Fig. 10a (compare Fig. 8e for the time

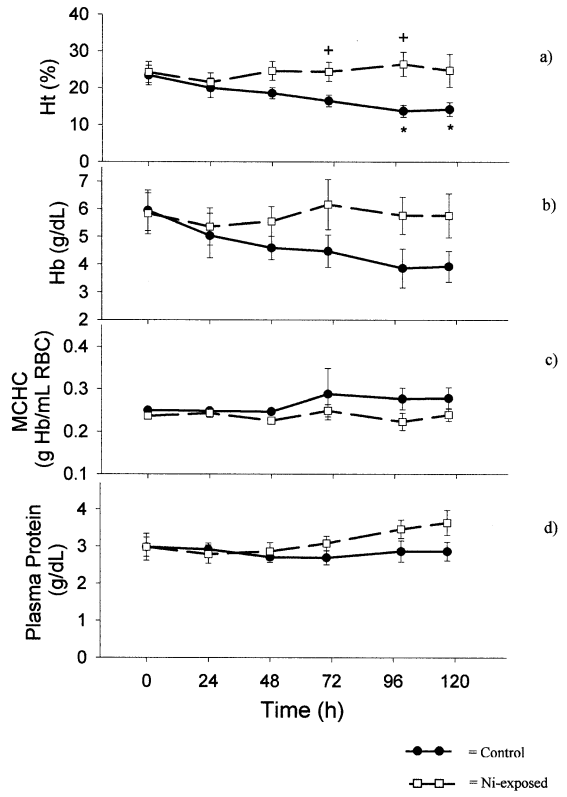

Fig. 9. Arterial hematological parameters obtained by cannulation of adult rainbow trout exposed to control conditions or 11.6 mg Ni l^{-1} over 117 h. (a) Hematocrit (Ht), (b) blood [Hb], (c) MCHC, (d) plasma protein concentration. Data are expressed as mean ± 1 S.E.M. ($n = 5$–8 control; $n = 4$–8 experimental). Asterisk '*' indicates significant difference ($P < 0.05$, one-way ANOVA plus two-sided Dunnett's post hoc test) from time 0 h mean. '+' Indicates significant difference ($P < 0.05$; two-tailed Student's t-test) from simultaneous control mean.

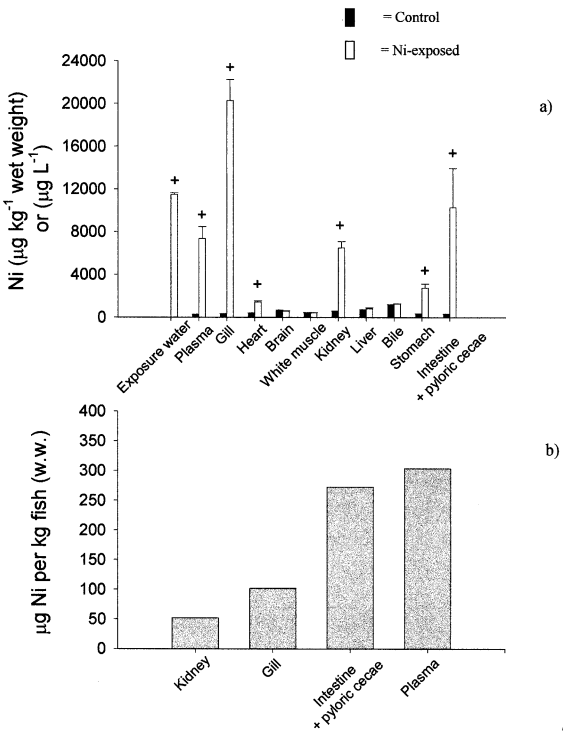

Fig. 10. (a) Ni accumulation in tissues of cannulated adult rainbow trout exposed to control or 11.6 mg Ni l^{-1} for 117 h. Ni level in the exposure water and in plasma is included for comparison. Ni concentrations in fluids are expressed as µg Ni l^{-1} (ppb), while Ni concentrations in tissues are expressed as µg Ni kg tissue^{-1} wet weight (ppb) for comparison. Data are expressed as mean (±1 S.E.M.) ($n = 9$ control; $n = 6$ experimental). '+' Indicates significant difference ($P < 0.05$; two-tailed Student's t-test) from time 0 h mean. The mean Ni level in control water (~4 µg Ni l^{-1}) is too low to appear in the plot. (b) Relative distribution of Ni among four tissues in a hypothetical 1 kg rainbow trout. Newly accumulated Ni in each tissue (µg Ni kg tissue^{-1}; see Fig. 10a) was multiplied by that tissue's relative proportion of total body weight to normalize individual tissue Ni burdens to a hypothetical 1 kg fish. Normalized accumulation is, therefore, expressed as µg Ni kg fish^{-1} for each tissue.

course of plasma Ni accumulation). Nickel accumulated significantly in the heart, stomach, kidney, intestine and gill—in increasing order of accumulation. Only the latter significantly exceeded plasma levels and the level in the exposure water. Ni levels in these tissues were 3.5, 8.1, 11.0, 33.8, and 60.0 times higher, respectively, in experimental versus control fish. Ni levels did not change significantly in brain, liver, bile, or white muscle.

4. Discussion

4.1. Ninety-six hour LC_{50}

The 96-h LC_{50} value of 15.3 mg Ni l^{-1} fits well with previous reports of rainbow trout LC_{50} values in waters of both lower and higher hardness. Hardness is well known to protect against Ni toxicity (USEPA, 1986). Buhl and Hamilton

(1991), and Nebeker et al. (1985) reported a 96-h LC_{50} for juvenile rainbow trout of 7.8 and 8.1–10.9 mg Ni l^{-1}, respectively. Both of these studies were run in softer water of only 40 mg l^{-1} $CaCO_3$ and are expectedly lower than our value obtained in moderately hard water of approximately 140 mg l^{-1} $CaCO_3$. Brown and Dalton (1970) reported a 48 h LC_{50} in hard water (240 mg l^{-1} $CaCO_3$) of 32.0 mg Ni l^{-1}.

4.2. Acute respiratory toxicity and acid/base disturbance

The physiological data presented in this study clearly indicate severe respiratory toxicity in adult rainbow trout acutely exposed to levels of waterborne $NiSO_4$ close to the 96-h LC_{50}. Ionoregulation was little affected. Indeed, severe, acute ionoregulatory toxicity with respect to Na^+, Cl^-, Ca^{2+}, or Mg^{2+} did not occur in either juvenile or adult rainbow trout during Ni exposure.

The progressive decreases in P_{aO_2} (Fig. 5a) and increases in P_{aCO_2} (Fig. 5c) over 99 h of Ni exposure indicate an increasing diffusion limitation at the gills over time. A sharp increase in P_{aCO_2} in experimental fish at 99 h caused a simultaneous respiratory acidosis (Fig. 5b). A significant increase in plasma lactate (99 h, Fig. 6a) also fits the scenario of decreased gas exchange. Reduced branchial diffusing capacity would decrease oxygen availability at the tissue level and constrain a decrease in oxidative metabolism, causing subsequent accumulation of lactate in the tissue and eventual transfer of lactate to the plasma. Although non-significant, a marked increase in plasma total ammonia (99 h, Fig. 7b) also suggests reduced branchial diffusing capacity.

Playle et al. (1989) found that respiratory toxicity in rainbow trout exposed to waterborne Al at pH 5.2 in soft water ($[Ca^{2+}] \cong 45$ μEq l^{-1}) led to a decrease in P_{aO_2} that was very similar to that caused by Ni in the present study. Impaired gas transfer in Al-exposed fish also led to P_{aCO_2} increases, albeit linear increases with time, as opposed to the delayed rise in P_{aCO_2} after 48 h of Ni exposure (Fig. 5c).

Other responses to decreased oxygen content of arterial blood include increased ventilation rate and stroke volume, and subsequent increase in ventilatory volume of water passed over the gill per unit time (Davis and Cameron, 1971; Perry and Wood, 1989). We observed increases in both ventilation rate and stroke volume, though only ventilation rate was quantified in the present study. Ventilation rate (Fig. 5e) increased steadily, though non-significantly, over time as P_{aO_2} fell (Fig. 5a). Cameron (1971) showed that rainbow trout blood at 15 °C and at a typical physiological CO_2 tension of ~2 torr was less than fully saturated with oxygen at oxygen tensions of less than about 60 torr. Based on similar in vitro oxygen dissociation curves of rainbow trout blood at 15 °C (Eddy, 1971), we estimate that during the respiratory acidosis at 99 h of Ni exposure, the arterial blood of experimental fish was, on average, 50% saturated with O_2, while arterial blood of the two most impacted experimental fish was below 30% saturation.

Physiologically, the observed hematological adjustments to Ni-induced hypoxia in experimental fish included increases in Ht (Fig. 9a) and a 30% reduction in spleen hemoglobin. These findings are consistent with the mechanism of α-adrenergically stimulated splenic contraction to release supplemental erythrocytes (and Hb) into the circulatory system to increase oxygen carrying capacity (Perry and Wood, 1989). Significantly decreased pH_a and elevated P_{aCO_2} at 99 h would have exacerbated hypoxia by decreasing the affinity (Bohr shift) and capacity (Root shift) of circulating Hb for O_2.

Strong evidence further supporting branchial impairment of gas exchange during Ni exposure is provided by early studies of Hughes and coworkers (Hughes and Perry, 1976; Hughes et al., 1979). These authors found that of cadmium, chromium, and nickel, only nickel had a concentration-dependent deleterious effect on the diffusing capacity of the gill and its ability to function in gas exchange. In that study, rainbow trout were exposed to 2.0 and 3.2 mg Ni l^{-1} (only about 17 and 27%, respectively, of the concentration used in series B in the present study) for 3.5 days in hard water of ~200 mg l^{-1} as $CaCO_3$. Following exposure to 3.2 mg Ni l^{-1}, the diffusing capacity

of the gill decreased by 85% and the harmonic mean thickness of the water/RBC barrier more than doubled. These perturbations were a result of thickening of non-cellular components of the epithelial layers of the secondary lamellae (Hughes et al., 1979). The authors hypothesized that concentration-dependent reduction in diffusing capacity would persist at higher concentrations. Nath and Kumar (1989) exposed tropical freshwater perch (*Colisa fasciatus*) to approximately 14 mg Ni l^{-1} in hard water (172 mg l^{-1} as $CaCO_3$) and observed massive damage to the gill architecture, including hypertrophy of respiratory and mucous cells, epithelial lifting, necrosis, hyperplasia of the respiratory epithelium, fusion of adjacent lamellae, and lamellar clubbing. Although a detailed analysis of overt signs of gill damage (i.e. excessive mucus secretion) was not undertaken in the present study, it will be one of the foci of future experiments.

Interestingly, in the present study plasma Ni rose almost linearly over 99 h of exposure (Fig. 8e), in contrast to the time course of plasma accumulation of other metals such as Ag, Cu, or Cd, which tend to saturate over time (Wood et al., 1996; Richards and Playle, 1999). Fig. 11 presents the relationship between plasma Ni concentration and arterial oxygen tension for all samples from Ni-exposed fish. The increase in plasma Ni exhibited a strong negative correlation ($P < 0.01$; Fig. 11) with the nearly linear decrease in P_{aO_2}, suggesting that branchial impairment of gas exchange is related to plasma Ni levels. In future studies, it will be of interest to determine whether the inhibition of gill diffusing capacity is caused by external Ni exposure, (i.e. waterborne Ni), internal Ni exposure, (i.e. bloodborne Ni), or both. Ni accumulated to the highest extent in the gills of experimental fish, reaching levels two times higher than any other tissue including plasma, and 60 times higher than control levels (Fig. 10). Measured gill Ni burden has been shown to be a good predictor of acute toxicity in fathead minnows (*Pimephales promelas*) exposed to Ni (Meyer et al., 1999). In that study, the gill burden of Ni associated with 50% mortality at 96 h (LA_{50}) was approximately 15 000 μg kg^{-1}(w.w.), or 250 μmol kg^{-1}. Interestingly, the gill Ni burden of experimental rainbow trout in the present study (survivors sampled at 117 h) was similar, approximately 20 000 μg kg^{-1} (w.w.), or 330 μmol kg^{-1} (Fig. 10a). Meyer et al. (1999) also showed that the LA_{50} of 250 μmol kg^{-1} was very consistent across a wide range of Ni concentrations and water chemistries.

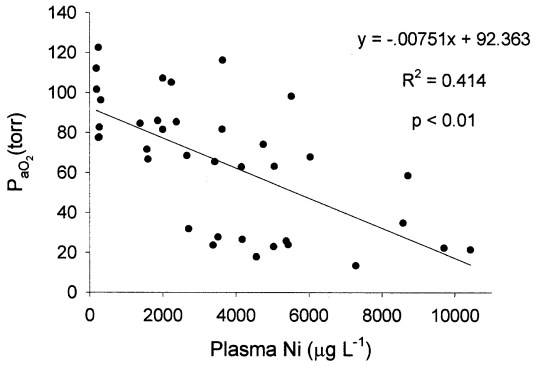

Fig. 11. Negative correlation between plasma Ni and P_{aO_2}. Data were obtained by cannulation of adult rainbow trout exposed to 11.6 mg Ni l^{-1} for 117 h, and represent all samples from Ni-exposed fish. The correlation is highly significant ($P < 0.01$).

4.3. Ca^{2+} homeostasis

With respect to Ca^{2+} homeostasis, no significant effect on Ca^{2+} uptake was observed in juvenile trout over 60 h of exposure to 15.6±0.3 mg Ni l^{-1} (Fig. 3). The only other study, to our knowledge, to measure Ca^{2+} uptake in fish by the use of ^{45}Ca in the presence of Ni also showed no significant effect on Ca^{2+} uptake by the brown trout (*Salmo trutta* L.) (Sayer et al., 1991). However, because that study employed a far different Ni concentration (300-fold lower [Ni] than the present study) and water hardness (50-fold lower [Ca] than the present study) detailed comparison with the present study is problematic. Guirguis et al. (1997) found a concentration-dependent Ni inhibition of Ca^{2+} uptake by the skin of intact *Xenopus laevis*. Nickel concentrations of 100 and 300 μM did not reduce Ca^{2+} uptake significantly, while 500 μM all but abolished Ca^{2+} uptake in *Xenopus* skin. The [Ni] used in our Ca^{2+} uptake experiments (15.6 mg $l^{-1} \cong$

260 μM), and the lack of significant inhibition of $J_{in}^{Ca^{2+}}$ that we observed at this concentration are in very good agreement with these data.

In the biochemical and electrophysiological literature, divalent Ni is widely used as a Ca^{2+} antagonist, blocking voltage-dependent calcium channels by binding to surface adsorption sites on the channels (Hille, 1984). The bulk of in vitro studies on Ca^{2+} antagonism have employed very high concentrations of Ni^{2+} ranging from 500 to 2000 μM (e.g. Nasu et al., 1993; Kamioka et al., 1994; Nutter and Adams, 1995). The results from the cannulation series B of the present study showed that despite 99 h of almost linear Ni accumulation in the plasma of rainbow trout, plasma [Ni] was only 110 μM (i.e. 6300 μg l^{-1}) at 99 h of exposure (Fig. 8e). Except for the gill, the highest tissue burden of Ni was in the intestine (and pyloric caeca) and reached 175 μmol kg^{-1} (w.w.) by 117 h (Fig. 10). These observed physiological levels are clearly lower than the levels typically used to study Ca^{2+} antagonism pharmacologically. Since the gill Ni burden was approximately 330 μmol kg^{-1} (w.w.) at 117 h (Fig. 10), however, we cannot rule out the possibility of some degree of branchial Ca^{2+} antagonism. Nevertheless, plasma Ca^{2+} levels in these fish were perfectly conserved, despite high gill Ni burdens (Fig. 8c).

4.4. Na^+ and Cl^- homeostasis

No marked impact on Na^+ or Cl^- unidirectional or net fluxes was observed over 48 h of exposure to the approximate 96-h LC_{50} level of Ni (Fig. 1). This finding is in direct contrast to the toxic mechanism of Na^+ antagonists such as Cu and Ag, which strongly inhibit Na^+ uptake by inhibition of Na^+–K^+-ATPase at the basolateral membrane of branchial epithelial cells (Lauren and McDonald, 1987; Morgan et al., 1997).

Juvenile trout (2 g) exposed to the 96-h LC_{50} level of Ni lost 7% of whole-body Na^+ over 48 h (Fig. 2). It should be noted that this percent loss is far different from the approximate 30% whole-body Na^+ loss that is indicative of lethal toxicity, and that is characteristic of exposure of rainbow trout to acutely toxic low pH (Wood, 2001). This 7% loss of Na^+ in juvenile trout represents a total loss of approximately 3.6 mmol kg^{-1} (Fig. 2), and can be explained in part by a contraction of plasma volume with unchanged plasma Na^+ concentration (Fig. 8a). The plasma protein data (Fig. 9d) provide indirect evidence for this phenomenon. As plasma protein concentration in control fish has been shown to be almost unaffected by repetitive blood sampling (Wood et al., 1988, 1996, present study), we assume that the 22% increase in plasma protein concentration (final vs. initial) in Ni-exposed animals (Fig. 9d) represented a contraction of plasma fluid volume of a reciprocal magnitude. This calculation ignores the minor change ($<4\%$) in plasma protein in control animals over the exposure period. Given a plasma Na^+ concentration of 145 mmol $l^{-1}$and a total intravascular volume of 60 ml kg^{-1} of fish (Bushnell et al., 1998), we calculate that adult trout would have experienced a Na^+ loss from the plasma compartment of approximately 1.9 mmol kg^{-1}. Therefore, we speculate that plasma Na^+ loss accounted for just over 50% of the whole-body Na^+ loss in juvenile fish. Although this calculation of plasma Na^+ loss is based on a plasma protein change over 99 h (compared with Na^+ loss in juvenile fish over 48 h of exposure), juvenile trout would likely be more sensitive, and were exposed to a concentration of Ni that was approximately 35% higher than adult trout.

Two scenarios might explain such an equal loss of plasma ions and water during Ni exposure. The first entails osmotic loss of plasma water to tissue, perhaps caused by the production of osmotically active lactate in muscle tissue associated with hypoxemia. This plasma fluid loss would have to be mirrored by increased diffusive loss of ions at the gill, perhaps caused by very high Ni burdens displacing Ca^{2+} from proteins within the tight junctions of the branchial epithelium. A second scenario entails simultaneous diuresis and natriuresis during Ni exposure. The kidney is considered to be the target organ of Ni toxicity in the mammalian literature and the site of the greatest Ni accumulation (Clary, 1975; Abdulwajid and Sarkar, 1983; Ghazaly, 1992), while physiological disruptions such as aminoaciduria and proteinuria have been documented following Ni exposure to

rats (Gitlitz et al., 1975). In the present study, Ni levels in the kidney were exceeded only by those in gills, intestine, and plasma (Fig. 10). Both of the above scenarios are amenable to future testing.

4.5. Tissue Ni accumulation

Tissue Ni accumulation data (Fig. 10a) and the relative proportion of total body weight represented by each tissue or body fluid (using values from Hogstrand et al., 2002) were used to calculate tissue specific Ni burdens and to estimate internal Ni distribution within certain tissues of the rainbow trout following 117 h of Ni exposure (Fig. 10b). Relative burdens were calculated only for tissues that significantly accumulated Ni over 117 h of exposure (Fig. 10a), and therefore, brain, white muscle, and liver are excluded from the analysis. While the plasma, gill, and kidney are understandable as Ni sinks, the large Ni accumulation by the intestine was unexpected in a waterborne exposure with no dietary component, and the physiological implications of intestinal Ni loading are unclear. We cannot eliminate the possibility that stress-induced drinking made a contribution to the intestinal Ni burden.

5. Conclusions

To our knowledge, the present study is the first to document the acute toxic mechanism of waterborne Ni in fish. In moderately hard water and at concentrations close to the 96-h LC_{50}, Ni acts as a respiratory toxicant, most likely causing sufficient gill damage to critically impair gas exchange leading to eventual suffocation. In this manner, Ni is quite similar to Al in soft water at moderately low pH, while clearly different from other waterborne metals (Cu, Ag, Cd, Zn) whose acute toxic mechanism of action is ionoregulatory. In the simplest sense, the BLM can be used with ionoregulatory toxicants to predict acute toxicity based on metal binding to ion transport sites at the gills (see DiToro et al., 2001). Therefore, this fundamental difference between Ni (and Al) and other metals presents a unique opportunity to investigate the applicability of the BLM to metals with acute mechanisms of action other than interference with ionoregulation at the gill surface. In the present study we have shown a strong correlation between plasma Ni levels and P_{aO_2} (Fig. 11), and possible pathological gill Ni levels following acute exposure (Fig. 10a and b). Whether these, or other, measured Ni parameters can be used to model acute respiratory toxicity by a BLM approach remains to be determined, though the recent data of Meyer et al. (1999) provide considerable encouragement in this regard.

Acknowledgements

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